#3

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Certificate

I, Patent Attorney Dipl.-Chem. Dr. Friedrich Baumbach

declare that I am competent in the German and English languages and I do hereby certify, that the annexed document is the best of knowledge and belief true and correct translation of the

PCT/DE98/03819 – WO 99/34824.

Declared at Berlin
this 28th day of September 2000.

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Claims: MDC 3804/05

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney's Docket: 0107-027

Applicant: Uwe Karsten et al.

Serial No.: N/A

Filed: June 29, 2000

For: TUMOR VACCINES FOR MUCI-POSITIVE CARCINOMAS

CLAIM FOR SMALL ENTITY STATUS
(Nonprofit Institution)

I hereby declare that I am an official empowered to act on behalf of the following nonprofit institution

Name of Organization: Max-Delbrück-Zentrum für Molekulare Medizin

Address: Robert-Rössle-Straße 10, 13125 Berlin, Germany

It is hereby declared that the rights in and to the above-idntified application or patent have not been assigned or licensed, and that there is no obligation to license or to assign such rights to any organization that, together with any and all of its affiliates, had more than 500 full- and part-time employees within the last 12 months.

The duty is acknowledged in this application or patent, to notify any change in status resulting in loss of entitlement to small entity status, prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date of which status as a small entity is no longer appropriate.

I hereby declare further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this declaration applies.

July 3, 2000

D. Ganten, Scientific Director

E. Jost, Admin Dirtector

MDC MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN Robert-Rössle-Str. 10 / 13125 Berlin

#6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Karsten, et al.

Ser. No. 09/606,910

Filed on June 29, 2000

For TUMOR VACCINES FOR MUCI-POSITIVE CARCINOMAS

Attorney's Docket 0107-027P

Box Amendment - No Fee
Hon. Commissioner of Patents and Trademarks
Washington DC 20231

PRELIMINARY AMENDMENT

Sir:

Prior to taking up this case for action, please enter the following preliminary amendment.

In the disclosure

Please enter the enclosed substitute disclosure and abstract.

Add the following abstract:

-- Abstract of the disclosure

A tumor vaccine containing synthetic peptides of a differing lenght derived mostly from human epithelial mucin MUC1 by glycosylation on threonine of the contained immunodominant PDTRPAP region.--

In the claims

Please replace originally filed claims 1-7 with the following new claims:

-- 8. A tumor vaccine containing synthetic peptides of a differing lenght which comprises glycosylating mostly but not exclusively human epithelial mucin MUC1 on threonine of the contained immunodominant PDTRPAP region.--

--9. The tumor vaccine of claim 8, wherein said synthetic peptides have a length of at least 20 amino acids.--

--10. The tumor vaccine of claim 8, wherein said glycosylation is carried out by a monosaccharide.--

--11. The tumor vaccine of claim 8, wherein said glycosylation is carried out by acetylgalactosamine (GalNAc).--

--12. The tumor vaccine of claim 8, wherein said glycosylation is carried out by a short-chain oligosaccharide.--

--13. The tumor vaccine of claim 8, wherein said glycosylation is carried out by the disaccharide Galβ-1, 3-GalNAc.--

--14. A process for combating tumor cells of mammary, colorectal, or pancreatic carcinomas by actiove specific immunization which comprises administering to a patient in need therefor a tumor vaccine of claim 8.--

REMARKS

Claims 8-14 are in the application.

The enclosed substitute specification is respectfully submitted not to contain new matter. A marked up copy of the translation is also enclosed to show the changes that went into the substitute specification.

Favorable consideration is respectfully urged.

Respectfully submitted

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Gabriel P. Katona
attorney of record

I hereby certify that this correspondence is deposited with the U S. Postal Service as first class mail, addressed as above, on October 18, 2000

Cynthia A. Pilato

Tumor vaccines for MUC1-positive carcinomas

Field of invention

The invention relates to tumor vaccines of a new type, based on the molecular structure of human epithelial mucin (MUC1). The invention can be used for the immunotherapy of carcinomas.

Background

Epithelial mucins are glycoproteins with repetitive amino acid sequences and a high proportion of carbohydrates which are partially bound to membranes, partially secreted and are to be found on many glandular epithelia. The epithelial mucin known best is the membrane-bound MUC1, described also as PEM, PUM, EMA, MAM-6, PAS-0 or episialine (Finn, O. et al., Immunol. Reviews 145:61, 1995) the extracellular part of which consists of a variable number of repeating units of 20 amino acids, the so-called tandem repeats. The MUC1 is not a tumor specific molecule *per se*; its suitability as tumor antigen is based on the fact that its carbohydrate portion is qualitatively and quantitatively changed in tumors (Burchell, J. and Taylor-Papadimitriou, J., Epith. Cell Biol. 2:155, 1993). Here, new epitopes appear which are detected by the immune system (humoral and cellular defense).

After operatively removing the primary tumor (or after a radiation or chemotherapy) one, as a rule, has to proceed on the assumption that tumor cells still remain in the body (minimal residual disease). These tumor cells which represent a potential danger, are combated by various endogenic mechanisms the efficiency of which may be intensified by an adjuvant immunotherapy. The most effective adjuvant immunotherapy is vaccination. Here, two prerequisites are present: first, a suitable target antigen (epitope) has to be present on the tumor cells, and second that it should be possible to prepare a form of vaccine that is immunogenically as strong as possible, most suitably in a synthetic form.

Non-glycosylated oligo-repeat peptides of MUC1 represent a suitable target antigen in a number of frequently occurring carcinomas (Apostolopoulos, V. and McKenzie, I.F.C., Crit. Rev. Immunol. 14:293, 1994). The immunodominant region of MUC1 is the PDTRPAP motif which occurs on each tandem repeat. However, experiments carried out so far to develop a vaccine on the basis of an individual tandem repeat have not been successful. According to the present state of knowledge a minimum length of the peptide which will be reached only in 3-5 tandem repeats is required for achieving the immunogenic conformation of the peptide (Fontenot, J.D. et al., J. Biomol. Struct. Dyn. 13:245, 1995).

Brief description of the drawing

The invention is described with reference to the drawing wherein

Fig. 1 shows bending of the anti-MUC1-antibody A76-A/C7 on the glycopeptides A1-A9 and A11-A12; and

Fig. 2 shows bending of the anti-MUC1-antibody MFO6 on the glycopeptides A9 and A11-A12.

Description of the invention

It is an object of the present invention to develop tumor vaccines on the basis of the molecular structure of human epithelial mucin MUC1 for combating tumor cells which remain in the body after other therapies.

In the immunological investigation of synthetic glycopeptides which correspond to a tandem repeat of the MUC1 there it was surprisingly detected that the glycosylation of threonine in the immunodominant PDTRPAP region with α-GalNAc significantly increases the antigenicity. So far we proceeded on the theory that this position is not glycosylated in native MUC1, because it was assumed previously that, as a rule, glycosylation hindered the

identification of peptide epitopes and the results of *in vitro* glycosylation experiments (Stadie, T. et al., Eur. J. Biochem. 229: 140 (1995). Latest investigations (Mueller, S., et al., J. Biol. Chem. 272:24780, 1997), however, showed that threonine may be well glycosylated *in vivo* in the PDTRPAP variant. From these latest results the conclusion was drawn that the antigenicity (and in this connection also the immunogenicity) of the MUC1 tandem repeat will be significantly increased by glycosylating threonine in the PDTRPAP variant by GalNAc or by a short oligosaccharide. Thus, the immunogenic conformation of the immunodominant region is already reached by an individual tandem repeat. The antigenicity of the glycosylated PDTRPAP variant in a monorepeat exceeds even that of the oligomeric non-glycosylated peptide.

This discovery develops tumor vaccine mostly but not exclusively from human epithelial mucin MUC1 various molecular sizes glycosylated on threonine of the PDTRPAP variant by GalNAc, or a short oligosaccharide. That objective is met by synthetic peptides of various lengths, suitably a synthetic peptide having a length of at least 20 amino acids, and modified by human epithelial MUC1 glycosylated threonine and containing the immunodominant PDTRPAP region. The glycolyzation can be suitably carried out by a monosaccharide, acetylgalactosamine (Ga1NAc), a short-chained oligosaccharide, and the disaccharide Ga1B-1, 3Ga1NAc.

The tumor vaccine of the present invention can be suitably administered to a patient against mammary, colorectal or pancreatic carcinomas.

The invention is explained in greater detail by reference to the following example.

Example

Antigenicity of synthetic, MUC1-derived glycopeptides

In the following experiment, the binding is investigated of monoclonal antibodies against the immunodominant PDTRPAP variant of the epithelial mucin to synthetic glycopeptides of this mucin in a solid-phase immunoassay (ELISA). The glycopeptides marked as A1 to A12 are indicated in the following Table. They correspond to an overlapping tandem repeat of MUC1 and contain 5 potential glycosylating sites (3 x threonine, 2 x serine); A1-A9 contain an additional alanine. The glycopeptides differ by the number and position of the glycosylating sites as specified in the Table. A1-A9 carry the Thomsen-Friedenreich (TF) antigen as glycan ß-D-Gal(1-3)α-D-GalNAc-O-R whereas A11 and A12 carry only α-GalNAc-O-R (the Tn antigen). The antibodies used are: **A76-A/C7** (mouse, IgG1, epitope: APDTRPAP) and **MFO6** (mouse, IgG1, epitope DTRPAP) (see: Rye, P.D., Price, M.R., eds., ISOBM TD-4 International Workshop on Monoclonal Antibodies against MUC1, Tumor Biol. 19, Suppl. 1, 1998).

Table: Synthetic glycopeptides; the peptide corresponds to the basic structure of the epithelial mucin (MUC1). The immunodominant region is underlined, as also shown in the drawing.

A: Glycosylation with TF:

A—H—G – V—T—S—A—P—D—T—R—P—A—P—G—S—T—A—P—P—A

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Peptide # glycosylated in position:

A1	5
A2	10
A3	17
A4	6
A5	16
A6	5, 17
A7	5, 16, 17
A8	5, 6, 16,17
A9	5, 6, 10, 16, 17

B: Glycosylation with Tn:

H –G—V—T—S—A—P—D—T—R—P—A—P—G—S—T—A—P—P—A

2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

Peptide # glycosylated in position:

A11	5,17
A12	5, 6, 10, 16, 17

The results show that the peptides glycosylated in position 10 with the two antibodies shown in the example bind significantly stronger than peptides not glycosylated in this position. Glycosylations in other positions have no influence. Substitution by Tn or TF is equal. The binding behavior demonstrated in this example is also shown by other MUC1 antibodies; yet, there are also exceptions. The increased antigenicity of the peptides glycosylated in position 10 can also be shown in inhibition experiments.

The results show that a glycosylation of the immunodominant region of the MUC1 peptide by means of Tn or TF significantly increases the antigenicity.

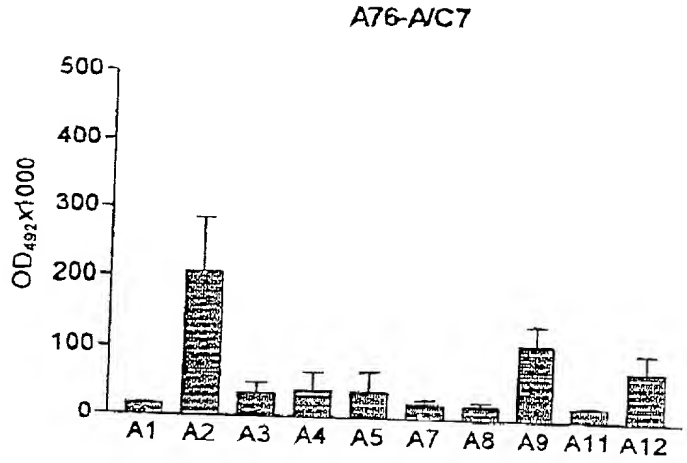
A76-A/C7
500
400
300
200
100
0
OD492X1000
A1
A2
A3
A4
A5
A7
A8
A9
A11
A12

Fig.1

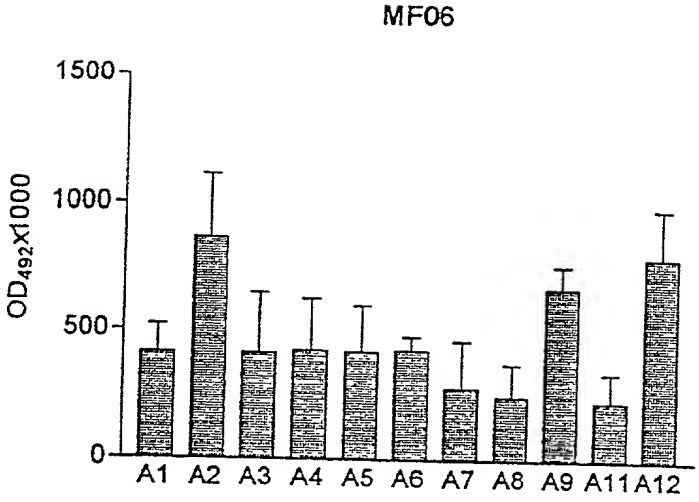
MF06
1500
1000
500
0
OD492X1000
A1
A2
A3
A4
A5
A6
A7
A8
A9
A11
A12

Fig. 2

#4

Attorney's Docket No.:0107-027

DECLARATION WITH POWER

I, the undersigned inventor hereby declare that my residence, post office address, and my citizenship are correctly stated below following my signature; that to the best of my knowledge I am the first, original and joint inventor of the invention described and claimed in the application for United States Letters Patent, having the title TUMOR VACCINES FOR MUCI-POSITIVE CARCINOMAS, the description of which was filed in the United States Patent and Trademark Office on June 29, 2000, and I state that I reviewed and understand the contents of the specification and claims and recognize my obligation pursuant to 37 C.F.R. 1.56 to disclose all information that is material to the patentability of this patent application. I hereby state that I authorized the filing of this application.

I hereby claim the benefit of priority under 35 U.S.C. 119 of German patent application No. 197 58 400.4, filed on December 30, 1997, and was subsequently filed as an international application designating the United States, under No. PCT/DE98/03819, on December 30, 1998.

I hereby appoint Gabriel P. Katona, Reg. No. 20,829; Sharon Blinkoff, Reg. No. 28,284; Henry Coleman, Reg. No. 32,559; and Neil R. Sudol, Reg. No. 31,669 to prosecute this application and to transact all business in the U.S. Patent and Trademark Office connected therewith, and I hereby request that all correspondence herein be directed to Gabriel P. Katona L.L.P., 708 Third Avenue, 14th floor, New York 10017; Telephone (212)370-4000; fax (212)370-7336.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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